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(54) Title: SOLUBLE TWO DOMAIN CD2 PROTEIN

(57) Abstract

A soluble CD2 peptide having an LFA-3 binding domain and antigenic epitopes recognized by antibodies raised against native CD2 on the surface of T cells and is capable of forming at least two intramolecular disulfide bonds. The peptide is capable of binding LFA-3, inhibiting CD2-mediated T cell activation and reacting with antibodies raised against CD2. Preferably, the soluble peptide comprises an amino acid sequence of 182 residues in length which is encoded by two extracellular segment exons of the gene encoding human CD2 glycoprotein.

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SOLUBLE TWO DOMAIN CD2 PROTEIN

Background

The human CD2 (T11) molecule is a 50KD surface glycoprotein expressed on >95% of thymocytes and virtually all peripheral T lymphocytes which mediates both adhesion between these cells and their cognate partners as well as subsequent activation events. Specific combinations of antibodies against the surface-bound molecule can activate IL-2 dependent T cell proliferation, helper T cell function and cytotoxicity by natural killer cells and cytolytic T lymphocytes (Meuer, S.C., et al., Cell 36:897-906 (1984); Brottier, P., et al., J. Immunol., 135:1624-1631 (1985); Siliciano, R.F., et al., Nature 317:428-430 (1985)) in the absence of cellular adhesion. In addition, thymocyte activation can be mediated via CD2 (Fox, D.A., et al., J. Immunol. 134:330-335 (1985); Denning, S.M., et al.., J. Immunol. 139:2573-2578 (1987)). The role of CD2 in approximation of T cells to various cell types including human thymic epithelial cells, B cells, target cells and sheep erythrocytes has been demonstrated to depend on direct interaction between CD2 and the broadly distributed human lymphocyte function-associated antigen 3 (LFA-3) surface glycoprotein or its sheep homologue, T11TS (Denning, S.M., et al.., J. Immunol. 139:2573-2578 (1987); Hunig, T., J. Exp. Med. 162:890-901 (1985); Hunig, T., et al., Nature 326:298-301 (1987); Shaw, S. et al., Nature 323:262-264 (1986); Selvaraj, P., et al., Nature 326:400-403 (1987); Vollger, et al., J. Immunol. 138:358-363 (1987)).

Biochemical analyses using specific monoclonal antibodies show that CD2 is T lineage-specific and exists on the cell surface in several differentially glycosylated forms (Howard, F.D., et al., J. Immunol. 126:2117-2122 (1981); Brown, M.H., et al., In Leucocyte Typing III ed McMichael, A.J., Oxford University Press pp. 110-112 (1987); Sayre, P.H., et al., Proc. Natl. Acad. Sci. USA 84:2941-2945 (1987)). CD2 cDNA clones predict a cleaved signal peptide of 24 amino acid residues, an extracellular segment of 185 residues, a transmembrane domain of 25 residues and a cytoplasmic region of 117 residues (Sayre, P.H., et al., Proc. Natl. Acad. Sci. USA 84:2941-2945 (1987); Sewell, W.A., et al., Proc. Natl. Acad. Sci. USA 83:8718-8722 (1986); Seed, B. and A. Aruffo, Proc. Natl. Acad. Sci. USA 84:3365-3369 (1987); Clayton, L.K., et al., Eur. J. Immunol. 17:1367-1370 (1987)). The corresponding genomic organization reveals a single exon encoding the signal peptide (less four residues), two exons encoding the extracellular segment, one exon encoding the transmembrane domain and charged membrane anchor segment, and one exon encoding the cytoplasmic region (Diamond, D.J., et al., Proc. Natl. Acad. Sci. USA 85:1615-1619 (1988)).

Summary of the Invention

This invention pertains to a soluble peptide having a lymphocyte function-associated antigen 3 (LFA-3) binding domain and antigenic epitopes recognized by antibodies raised against native

surface CD2 on resting T lymphocytes. The soluble peptide is capable of forming at least two intramolecular disulfide bonds and binds the surface-bound CD2 legand, LFA-3. The soluble peptides of this invention exist as monomers in an aqueous medium. Preferably, the soluble peptides have an amino acid sequence encoded by the two extracellular segment exons of the gene encoding human CD2 glycoprotein. Most preferably, the soluble peptides have about 182 amino acid residues encoded by the two extracellular segment exons of the gene encoding human CD2 glycoprotein. Soluble peptides of this invention can be made by enzymatic fragmentation, peptide synthesis, or recombinant DNA technology. They can be used to block T cell function which is dependent upon antigen activation.

Brief Description of the Drawings

Figure 1 shows the amino acid sequence and exon organization of human CD2.

Figure 1a shows the DNA sequence of human CD2 glycoprotein.

Figure 2 shows the structure of recombinant soluble $T11_{ex2}$, native CD2 and its genomic organization.

Figure 3 shows the construction of expression plasmid for production of the CD2 external segment molecule $T11_{ex2}$.

Figure 4 shows SDS-PAGE analysis of purified, radioiodinated and endoglyosidase digested $T11_{ex2}$.

Figure 5 shows equilibrium sedimentation data.

Figure 6 shows the circular dichroism spectra of $T11_{ex2}$.

Figure 7 shows the competitive inhibition of radioiodinated $T11_{ex2}$ binding of JY cells.

Figure 8 shows the saturation binding of $T11_{ex2}$ to JY cells and Scatchard analysis.

Detailed Description of the Invention

This invention pertains to a soluble peptide having a lymphocyte function-associated antigen 3 (LFA-3) binding domain and antigenic epitopes recognized by antibodies raised against native surface CD2 on resting T lymphocytes. The soluble peptide is capable of forming at least two intramolecular disulfide bonds and binds the surface-bound CD2 ligand, LFA-3. Specifically, the antigenic epitopes which are localized on the peptide are $T11_1$, $T11_2$ and $T11_3$. The soluble peptides are further capable of inhibiting CD2-mediated T cell activation and are soluble in aqueous medium and exist as monomers in the same.

In one embodiment, the soluble peptides have an amino acid sequence encoded by the two extracellular segment exons of the gene encoding human CD2 glycoprotein. The soluble peptides are capable of binding the surface-bound CD2 ligand, LFA-3, and can react with antibodies raised against native CD2 on the surface of human T cells. The soluble peptides are further capable of inhibiting CD2-mediated T cell activation. The peptides exist as monomers in an aqueous medium.

In the preferred embodiment, the soluble peptide comprises about 182 amino acid residues

encoded by the two extracellular segment exons of the gene encoding human CD2 glycoprotein. The soluble peptide is monomeric in aqueous medium and comprises four cysteine residues located in the carboxy-terminal region of the amino acid sequence. The peptide is capable of forming at least two sets of intramolecular disulfide bonds between the amino-terminal cysteines and the carboxy-terminal cysteines. Preferably, the peptide has the following amino acid sequence:

amino

	Lys	Glu	Ile	Thr	Asn	Ala	Leu	Glu	Thr	Trp
11	Gly	Ala	Leu	Gly	Gln	Asp	Ile	Asn	Leu	Asp
21	Ile	Pro	Ser	Phe	Gln	Met	Ser	Asp	Asp	Ile
31	Asp	Asp	Ile	Lys	Trp	Glu	Lys	Thr	Ser	Asp
41	Lys	Lys	Lys	Ile	Ala	Gln	Phe	Arg	Lys	Glu
51	Lys	Glu	Thr	Phe	Lys	Glu	Lys	Asp	Thr	Tyr
61	Lys	Leu	Phe	Lys	Asn	Gly	Thr	Leu	Lys	Ile
71	Lys	His	Leu	Lys	Thr	Asp	Asp	Gln	Asp	Ile
81	Tyr	Lys	Val	Ser	Ile	Tyr	Asp	Thr	Lys	Gly
91	Lys	Asn	Val	Leu	Glu	Lys	Ile	Phe	Asp	Leu
101	Lys	Ile	Gln	Glu	Arg	Val	Ser	Lys	Pro	Lys
111	Ile	Ser	Trp	Thr	Cys	Ile	Asn	Thr	Thr	Leu
121	Thr	Cys	Glu	Val	Met	Asn	Gly	Thr	Asp	Pro
131	Glu	Leu	Asn	Leu	Tyr	Gln	Asp	Gly	Lys	His
141	Leu	Lys	Leu	Ser	Gln	Arg	Val	Ile	Thr	His
151	Lys	Trp	Thr	Thr	Ser	Leu	Ser	Ala	Lys	Phe
161	Lys	Cys	Thr	Ala	Gly	Asn	Lys	Val	Ser	Lys
171	Glu	Ser	Ser	Val	Glu	Pro	Val	Ser	Cys	Pro
181	Glu	Lys								

carboxy

The soluble peptide of about 182 amino acid residues in length has an LFA-3 binding domain and comprises the antigenic epitopes $T11_1$, $T11_2$ and $T11_3$ which are localized on the soluble peptide. The epitopes, which are localized on this peptide are antigenic determinants which are recognized by antibodies against the native surface CD2 structure on resting T lymphocytes. Thus, the peptides of this invention can be used to inhibit CD2-mediated T cell activation.

The above amino acid sequence corresponds with a portion of the naturally occurring extracellular domain of CD2 that is capable of binding LFA-3, inhibiting CD2-mediated T cell activation and reacting with antibodies raised against native CD2 on the surface of human T cells. Amino acid sequences embraced by this invention include analogous or homologous sequences which encode proteins capable of binding LFA-3, inhibiting CD2-mediated T cell activation and reacting with antibodies raised against native CD2. In addition, the peptide structure can be modified by deletions, additions, inversions, insertions or substitutions of one or more amino acid residues in the sequence to yield peptides having the characteristics of the peptides of this invention. All such modifications of the above amino acid sequence are embraced by this invention without essentially detracting from the properties of the peptide, i.e., the capacity of the peptide to bind LFA-3, inhibit CD2-mediated T cell activation and react with antibodies raised against native CD2 on the surface of human T cells.

Naturally occuring allelic variations and modifications are included within the scope of the invention so long as the variation does not substantially reduce the ability of the peptide to bind LFA-3 and inhibit CD2-mediated T cell activation.

The soluble peptides of this invention carry epitopes recognized by antibodies against native surface CD2 structure on resting T lymphocytes and interacts specifically with the surface-bound CD2 ligand, LFA-3. Additionally, the soluble peptide exists as a monomer in aqueous medium and includes a proteolytically-resistant amino-terminal fragment encoded by the first extracellular segment exon of the gene encoding human CD2 glycoprotein. The proteolytically resistant fragment comprises about 100 amino acid residues which correspond with the amino-terminal portion of the extracellular domain of human CD2 glycoprotein and is capable of inhibiting CD2-mediated T cell activation. The 100 amino acid fragment has been described in U.S. Patent Application Serial No. 07/293,330 filed January 4, 1989, by Reinherz et al., the teachings of which are incorporated herein by reference. The entire amino acid sequence and exon organization of human CD2 glycoprotein is shown in Figure 1. This amino acid sequence of human CD2 was deduced from cDNA (Sayre, P.H., et al., Proc. Natl. Acad. Sci. USA 84:2941-2945 (1987); Sewell, W.A., et al., Proc. Natl. Acad. Sci. USA 83:8718-8722 (1986); Seed, B. and A. Aruffo, Proc. Natl. Acad. Sci. USA 84:3365-3369 (1987)) and genomic clones (Diamond, et al., Proc. Natl. Acad. Sci. USA 85:1615-1619 (1988)).

This invention also pertains to an isolated DNA sequence that encodes a soluble monomeric human CD2 peptide having an LFA-3 binding domain and antigenic epitopes recognized by antibodies raised against native CD2 on the surface of human T cells and is capable of forming at least two intramolecular disulfide bonds. Specifically, the antigenic epitopes are $T11_1$, $T11_2$ and $T11_3$. The peptide that is encoded by the isolated DNA of this invention and is capable of binding LFA-3, inhibiting CD2-mediated T cell activation and reacting with antibodies raised against native CD2 on the surface of human T cells. In a preferred embodiment, the isolated DNA sequence encodes a soluble peptide having an amino acid sequence encoded by the two extracellular segment exons of the gene encoding the human CD2 glycoprotein. The encoded peptide is capable of binding LFA-3, inhibiting CD2-mediated T cell activation and reacting with antibodies raised against native CD2. Preferably, the isolated DNA of the invention encodes the 182 amino acid sequence shown above or substantial coding equivalent thereof. The DNA sequence can be modified by deletion, insertion or substitution of nucleotides to yield peptides which exhibit substantially the same properties of the above peptide of about 182 amino acid residues. All such modifications of the DNA sequence are within the scope of this invention so long as the DNA sequence encodes a soluble peptide that is capable of binding LFA-3 and inhibiting CD2-mediated T cell activation. The isolated DNA sequences of the invention can be made

using recombinant DNA technology or chemically synthesized. The DNA sequence for CD2 glycoprotein is shown in Figure 1a.

This invention further pertains to a recombinant expression vector comprising the DNA sequence encoding a soluble, monomeric human CD2 peptide having an LFA-3 binding domain and the antigenic epitopes $T11_1$, $T11_2$ and $T11_3$. The antigenic epitopes are recognized by antibodies raised against native CD2 on resting T cells and interacts specifically with LFA-3. The encoded soluble protein is capable of forming at least two intramolecular disulfide bonds. Alternatively, the expression vector comprises a DNA sequence encoding a soluble CD2 protein encoded by the two extracellular segment exons of the gene encoding human CD2 which is capable of inhibiting CD2-mediated T cell activation and LFA-3 binding. Preferably, the expression vector is a baculovirus transfer vector and comprises a DNA sequence which encodes a peptide of about 182 amino acid residues as shown above. Other vectors, however, may be used, including prokaryotic and eukaryotic expression systems. Modifications of the peptide in which amino acid residues have been deleted, inserted or substituted without essentially detracting from the properties of the peptide are embraced by the invention. The invention further pertains to cells transformed with the above expression vector.

Soluble peptides of this invention can be made by enzymatic fragmentation of human CD2 glycoprotein

or a portion thereof, by peptide synthesis or recombinant DNA technology. Preferably, the soluble CD2 peptides will be produced by inserting DNA encoding a peptide sequence which is capable of binding LFA-3 and inhibiting CD2-mediated T cell activation (e.g., CD2 DNA which represents the desired amino acid sequence of the extracellular domain of CD2) into an expression vector. The transformed cells then express the soluble human CD2 peptide encoded by the two extracellular segment exons of the gene encoding the human CD2 glycoprotein. The peptide expressed by the transformed cells is capable of binding LFA-3 and inhibiting CD2-mediated T cell activation. In addition to genetic engineering techniques for synthesizing soluble peptides of the invention, the soluble peptides can be synthesized directly by procedures of chemical protein synthesis. For example, the above 182 amino acid sequence or modified equivalent thereof can be synthesized by the solid phase procedure of Merrifield.

This invention further pertains to a method of inhibiting T cell activation, comprising the step of administering to a patient, a soluble peptide having an LFA-3 binding domain, antigenic epitopes recognized by antibodies raised against native CD2 on the surface of human T cells and is capable of forming at least two intramolecular disulfide bonds. In another embodiment, a patient is administered a solution containing a soluble peptide having an amino acid sequence encoded by the two extracellular

segment exons of the gene encoding human CD2 glycoprotein. The peptide is capable of binding LFA-3, inhibiting T cell activation and reacting with antibodies raised against the native CD2 protein. Preferably, the soluble peptide comprises a sequence of about 182 amino acid residues which corresponds with the portion of the extracellular domain of human CD2 glycoprotein or fragment thereof which is capable of binding LFA-3 and inhibiting CD2-mediated T cell activation. In one embodiment of the method, the soluble peptide can be administered intravenously.

The soluble CD2 peptides of this invention generally will bind to human lymphocytes and human red blood cells which express a homolgous set of surface structures. The soluble peptides are also capable of competing with the naturally-present CD2 on the surface of a human lymphocyte, thus interfering with the ability of the lymphocyte to make contact with its target cells (if the lymphocyte is a cytolytic cell), or with macrophages having CD2 binding structures which permit the cell-to-cell contact necessary for lymphocyte proliferation. To test a soluble peptide for the ability to inhibit lymphocyte proliferation, or the cytotoxic effector function, the soluble peptide is contacted with the lymphocytes prior to stimulation with mitogen, and degree of proliferation is measured, using standard techniques, and the result compared to a control in which the soluble peptide was not used.

The soluble peptides of this invention can be used in a variety of diagnostic and therapeutic applications in which the CD2 surface glycoprotein is expressed on the surface of many human T cell malignancies, e.g., T cell leukemias and lymphomas. In addition, autoimmune diseases, e.g., rheumatoid arthritis and Systemic Lupus Erhthmatosis (SLE), are characterized by the presence in the blood and lymph of large numbers of CD2-bearing T cells. Rapid cell turnover in these disease states can cause the shedding of the CD2 molecule into the bloodstream.

The CD2 soluble peptides of this invention can be used as an immunogen to produce polyclonal or monoclonal anti-CD2 antibodies, using conventional techniques. These antibodies can be labeled with any conventional label, e.g., radioisotopes, and used in conventional immunoassay methods to measure serum CD2 levels and thus monitor patients having T cell associated diseases. Particularly sensitive ELISA-type assays will employ two anti-CD2 antibodies, each to a different antigenic determinant on the surface of CD2, in a sandwich format.

The disease states which can be treated using the soluble peptides of this invention include medical conditions characterized by unwanted activity of the immune system which results in excess T cell activation, which plays a key role in the amplification of the immune response. These conditions include SLE; juvenile onset diabetes; multiple sclerosis; allergic conditions; inflammatory conditions such as exzema, ulcerative

colitis, inflammatory bowel disease, and Crohn's disease; and allograft rejection (e.g., rejection of a transplanted heart or kidney). The soluble CD2 peptides compete with the surface-bound CD2 for its ligand on target cells thus dampening immune response amplification. The soluble CD2 peptide admixed with a pharmaceutically acceptable carrier substance such as saline, is administered intraveneously to a human patient in an effective amount, e.g., 20 μg to 500 μg per kg body weight. Preferably, the soluble peptide admixed with a pharmaceutically acceptable carrier comprises about 182 amino acid residues which correspond to a portion of the extracellular domain of CD2 that is capable of binding LFA-3 and inhibiting CD2-mediated CD2 activation. For some conditions, a soluble CD2 peptide can be administered directly to the site where needed most; for example, a soluble CD2 peptide can be injected directly into the inflammed joint of a human patient suffering from rhematoid arthritis.

This invention is further illustrated by the following Exemplification.

EXEMPLIFICATION

The term Tll_{ex2}, as referred to in the Exemplification, is defined herein to be a soluble protein having an amino acid sequence encoding the two extracellular segment exons and a codon (182-Lys) derived from the transmembrane domain exon of the CD2 glycoprotein. The entire amino acid

sequence for CD2 has been described. See U.S. Patent Application Serial No. 932,871, filed November 18, 1986, by Reinherz et al., the teachings of which are incorporated herein by reference.

Baculovirus Transfer Vector Plasmid Construct for $T11_{ex2}$

The plasmid pAc373/$T11_{ex2}$ was constructed by digestion of pGEM-4-S1, a pGEM derivative containing a 950 bp fragment of the CD2 cDNA PB2 (Sayre et al., Proc. Natl. Acad. Sci. USA 84:2941-2945 (1987)) with PvuII, which digests the cDNA at nucleotide position 628 near the start of the transmembrane region. A double-stranded synthetic oligonucleotide linker:

CTGTCCAGAGAAATAAGGATCCT
GACAGGTCTCTTTATTCCTAGGA

containing the last base for the Ser-178 codon, codons for Cys-Pro-Glu-Lys, a stop codon and a BamHI restriction site was synthesized and ligated to the blunt PvuII ends. After BamHI digestion, the insert was cloned into the BamHI site of the pAc373 baculovirus transfer vector (Smith et al., Annu. Rev. Immuno. 2:319-333 (1985)). All restriction enzymes were produced from New England Biolabs (Beverly, MA).

Recombinant protein production

Transfer of the $T11_{ex2}$ sequences from the plasmid vector to the AcNPV genome to generate recombinant baculovirus $T11_{ex2}$-AcNPV was ac-

complished essentially as described (Smith et al., Proc. Natl. Acad. Sci. USA, 82:8404-8408 (1985); Hussey et al., Nature, 331:78-81 (1988). Metabolic labeling with ^{35}S-cysteine of $T11_{ex2}$-AcNPV-infected SF9 cells was carried out as described (Hussey et al., Nature 331:78-81 (1988). Metabolically labeled culture supernatants were harvested, microfuged for 10 min. and subjected to immunoprecipitation for 16 h at 4°C with a monoclonal anti-CD2 antibody (anti-$T11_1$, 3T4-8B5) linked to Affigel-10 (Biorad) beads (10 mg monoclonal antibody/ml gel). After immunoabsorption the beads were washed twice with lysis buffer and bound material eluted with 0.1 M glycine, HCl buffer, pH2. Eluates were analyzed by SDS-PAGE in 12.5% gels. Large protein production was performed as described (Hussey et al., Nature 331:78-81 (1988)) except that proteins were purified over an anti-$T11_1$ (3T4-8B5) immunoaffinity column.

Protein microsequencing

Proteins were prepared for microsequencing by electrophoresis on 12.5% polyacrylamide gels, followed by electroblotting onto polyvinylidene difluoride membranes according to the method of Matsudaira, J. Biol. Chem. 262:10035-10038 (1987). After visualization with Coomassie blue, stained bands were excised and loaded onto an Applied Biosystems (Foster City, CA) 470A sequencer and sequenced using the O3RPTH program.

Endoglycosidase F digestion of $T11_{ex2}$

1 μg samples of purified $T11_{ex2}$ dialyzed against PBS were incubated in Endo-F buffer (0.1 M sodium phosphate, 1% NP-40, 1% 2-mercaptoethanol, 50 mM EDTA, 1 mM phenylmethyl-sulfonyl fluoride, pH 6.1) in the presence of 0.7 units Endo-F (NEN) in 7 μl reaction volumes. Digestion was arrested at the indicated times by addition of 15 μl SDS sample buffer, boiling for 5 min. and freezing at -20°C prior to analysis by 12.5% SDS-PAGE.

Equilibrium sedimentation

Sedimentation studies were performed using the short column, high speed meniscus-depletion method of Yphantis Biochem. 3:297-317 (1964); and Richardson et al., Biochem. J. 135:87-92 (1973)). Standard double-sector cells, equipped with sapphire windows, 4 mm solution column lengths and a temperature of 21°C were used.

Papain digestion

Papain (32 ng) was added to 8 μg samples of $T11_{ex2}$ at 0.5 mg/ml in PBS containing 10 mM DTT for an enzyme:protein ratio of 1:250. Samples were incubated at 37°C for 15, 30, 45 or 60 min. Digestions were stopped by the addition of SDS buffer and boiling for 5 min. Samples were electrophoresed on a 12.5% polyacrylamide gel and stained with Coomassie blue.

Circular dichroism

Far ultraviolet CD spectra were obtained on an Instruments SA Jobin Yvon circular dichrograph calibrated with (+)10-camphorsulfonic acid and epiandosterone. Measurements were taken at 25, 50 and 80°C +/-0.1°C in 10 mM sodium phosphate pH 7.2 in a 1 mm cell. All spectra represent an average of 3 to 5 individual spectra with data taken at 0.5 mm intervals using a 10 second response time for each point. Protein concentrations were determined by quantitative amino acid analysis of aliquots taken from the sample cells.

Rosette inhibition assay

Jurkat cells were washed with SMEM/2% FCS (wash) and resuspended at 10^7/ml. Sheep erythrocytes were washed twice in HBSS and resuspended in wash to 5% v/v. 10 µl of sheep erythrocytes were aliquoted into 12-75 mm plastic tubes and 100 µl wash, $T11_{ex2}$ protein or control soluble CD4 $T4_{ex1}$ protein (Hussey et al., Nature 331:78-81 (1988) was added, followed by incubation at 4°C for 30 min. Subsequently, 20 µl of Jurkat cells were added after 5 min., centrifuged at 800 rpm in a Sorvall RT6000, followed by incubation at 4°C for 1 h. The cell-mixture was gently resuspended and mounted on glass slides with cover slips and rosette formation assessed on a Zeiss photomicroscope.

Radiolabeled $T11_{ex2}$ binding assays

The purified recombinant soluble CD2 extracellular domain molecule $T11_{ex2}$ was radioiodinated as follows: 50 µl $T11_{ex2}$ (1 mg/ml) dialyzed against PBS was labeled with 10 µl immobilized lactoperoxidase/ glucose oxidase (Enzymobeads; Biorad Laboratories, Richmond, CA) in 40 mM sodium phosphate, pH 7.2, 0.4% glucose and 1 mCi ^{125}I for 5 min. After quenching the reaction for 20 min. with 20 mM sodium iodide and 0.02% sodium azide, 20 µl FCS was added and the free iodine separated on a 1 ml Bio-Gel P-6 column (Biorad Laboratories) conditioned with 0.2M sodium phosphate pH 7.2, 10% FCS and run in the same buffer.

Cold competition studies: 5×10^6 cpm radiolabelled $T11_{ex2}$ (2.8×10^8 cpm/nmole) was added at 0.1 µM to 1.8×10^6 JY cells overlayed onto 0.2 ml of a 1.5:1 mixture of dibutyl phthalate:dioctyl phthalate (Aldrich Chemical Co., Milwaukee, WI) in 0.5 ml plastic tubes as described in Teshigawara et al., J. Exp. Medicine 165:223-238 (1987). Increasing concentrations of unlabelled $T11_{ex2}$ or $T4_{ex1}$ were added in a final volume of 200 µl in RPMI 1640/10% FCS. After 1 h incubation at 4°C, the tubes were centrifuged (8,500 g for 1 min.), the tips of the tubes containing the cell pellets were cut, and the cell-bound and free radioactivity were determined in a gamma counter. To some tubes, anti-LFA-3 antibody TS2/9 (generously provided by Dr. Timothy Springer, Dana Farber Cancer Institute)

was added at 50 μg/ml as a separate determination of nonspecific binding. This concentration of anti-LFA-3 was independently shown to give maximal inhibition of $T11_{ex2}$ binding. Specific activity was calculated using a MW for $T11_{ex2}$ of 30,000. Protein concentrations were determined by quantitative amino acid analysis of standard $T11_{ex2}$ samples. Subsequent samples were compared to standards by Coomassie blue staining of two-fold dilutions of standard and test samples run on the same gel and analyzed by densitometry.

Saturation binding studies: Increasing concentrations of radiolabelled $T11_{ex2}$ (1.31×10^7 cpm/nmole) were added to 2.6×10^6 JY cells in the presence or absence of 50 μg/ml anti-LFA-3 antibody to determine nonspecific binding. Binding was carried out as above and the dissociation constant determined by Scatchard analysis after subtraction of nonspecific binding determined in the presence of anti-LFA-3.

RESULTS

Production and purification of $T11_{ex2}$

A construct for expression of a soluble fragment of CD2 that included all the residues encoded by the leader and two extracellular segment exons was designed (Figure 2, exons 1-3).

Figure 2 shows a comparison of the 182 extracellular CD2 amino acids comprising $T11_{ex2}$ (top) to CD2 protein structure (middle). The positions of cysteine residues (C), carbohydrate addition sites

(CHO), the CD2 leader segment (L) and the CD2 transmembrane domain (TM) are indicated. In the CD2 gene (bottom), exon 1 corresponds to CD2 amino acid residues -24 to -4, exon 2 to residues -4 to 104, exon 3 to residues 104 to 181, exon 4 to residues 181 to 222, and exon 5 to residues 222 to 327 (Diamond et al., PNAS USA 85:1615-1619 (1988).

The plasmid pAc373/T11$_{ex2}$ was constructed and encodes 182 amino acids of the predicted CD2 external segment including all of the residues derived from the two extracellular exons (Figure 2) and part of one codon (for Glu-181) and all of a second codon (for Lys-182) derived from the transmembrane domain exon. This construction, thus, includes all four extracellular cysteine residues located in domain II of CD2 and thereby avoids problems associated with intermolecular disulfide exchange observed with a previous construction (Richardson, et al., Proc. Natl. Acad. Sci USA 85:5176-5180 (1988)).

Plasmid pAc373/T11$_{ex2}$ was used to co-transfect SF9 with AcNPV baculoviral DNA. Recombinant baculovirus, termed T11$_{ex2}$-AcNPV, were selected, purified and used to infect small-scale cultures for metabolic labeling. Immunoprecipitation of radiolabelled supernatants with anti-T11$_1$ (3T4-8B5), an anti-CD2 specific monoclonal antibody (Meuer et al., Cell 36:897-906 (1984), verified that T11$_{ex2}$-AcNPV directed the production of a recombinant CD2 molecule in SF9 cells (data now shown). T11$_{ex2}$-AcNPV was therefore used to infect liter cultures for the production of large amounts of protein. T11$_{ex2}$ protein was purified from infected

cell supernatants by affinity chromtography on an anti-T11 column.

The construction of expression plasmid for production of the CD2 external segment molecule $T11_{ex2}$ is shown in Figure 3. The plasmid pGEM-4-S1 carries a 950 bp fragment of the CD2 cDNA. After digestion of pGEM-4-S1 with PvuII, ligation of the double-stranded linker and further digestion with BamHI, the cDNA insert was isolated and ligated into the BamHI-digested baculoviral transfer vector pAc373. The resulting plasmid pAc373/$T11_{ex2}$ encodes 182 amino acids of the mature CD2 extracellular segment. The promoter for the polyhedrin gene in the pAc373 transfer vector is shown by the black box and the polyhedrin gene is indicated by the open box. The 950 bp CD2 coding fragment in pGEM-4-S1 is shown in a stippled box. The position of the T7 polymerase promoter in the pGEM vector is shown.

Biochemical characterization of $T11_{ex2}$

Figure 4 shows the purification, radioiodination and endoglycosodase digestion of $T11_{ex2}$. Lanes a-d contain 1 λg $T11_{ex2}$ purified from large scale cultures of SF9 cells infected with $T11_{ex2}$-AcNPV was analyzed by Coomassie staining on a 12.5% polyacrylamide gel in the presence of 50 mM DTT (lane a) or in nonreducing conditions (lane b). An aliquot of $T11_{ex2}$ radioiodinated with solid-phase lactoperoxidase/glucose oxidase was analyzed on the same gel in the presence (lane c) or absence (lane d) of 50 Mm DTT by autoradiography. Lanes e-k

contain 1 μg purified $T11_{ex2}$ which was digested with 9.7 units Endo-F for varying amounts of time and the reaction stopped by the addition of SDS sample buffer prior to SDS-PAGE analysis and Coomassie staining. Lane e contains no enzyme; f, simultaneous addition of enzyme and sample buffer; g-j, 1 min, 5 min, 1 h, 18 h digestion; k, 0.7 units Endo-F alone.

$T11_{ex2}$ migrates as a well-demarcated doublet in both reducing and non-reducing conditions in SDS-PAGE (Figure 4, lanes a and b). Two well-separated bands at 30-31KD are seen in the presence of 50 mM DTT (lane a), which migrate at 27-28KD in the absence of reducing agent (lane b). The clear-cut decrease in electrophoretic mobility after reduction with DTT strongly indicates that $T11_{ex2}$ contains intrachain disulfide bridges; it does not form interchain bridges. Although not shown, micro-sequencing analysis of ^{35}S-cysteine labeled peptides verifies that there are two sets of intrachain disulfide bonds in $T11_{ex2}$ between the amino-terminal cysteines and carboxy-terminal cysteines.

To investigate the difference between the two bands representing $T11_{ex2}$, 160 pmole of purified protein was separated by SDS-PAGE and blotted onto a PVDF membrane (Matsudiara, J. Biol. Chem. 262:10035-10038 (1987)). The upper and lower bands were cut separately from the membrane for amino-terminal sequencing. Each band yielded the CD2 amino-terminal sequence, suggesting that they differ from one another by post-translational modification.

As shown in Figure 4, endoglycosidase digestion generates at least five distinct bands. After short digestion times, two new lower molecular weight species are generated (lanes f-h). Some glycans on Tll_{ex2} are apparently quite susceptible to digestion since even after simultaneous addition of enzyme and SDS sample buffer, these new species are generated (lane f). After 1 h digestion, most of the Tll_{ex2} protein is digested to a 25KD species (line i); overnight digestion results in complete digestion to a single band at 25KD (lane j). Note that the band of approximately 45KD size represents the Endo-F enzyme since it appears in lane k, where an equivalent amount of enzyme alone has been analyzed. The origin of the faint band at 48KD in lane j is unclear.

To determine whether Tll_{ex2} exists as a non-covalently linked multimer in aqueous solution, it was subjected to equilibrium sedimentation by the high-speed meniscus depletion method (Yphantis, Biochem. 3:297-317 (1964) in both aqueous and dissociating conditions. As shown in Figure 5, the calculated molecular weights for both conditions are very similar (25.3KD in aqueous solution vs. 24.7KD in dissociating conditions). This result demonstrates that Tll_{ex2} exists as a monomer in solution.

Figure 5 shows the equilibrium sedimentation analysis as a plot of log (fringe displacement) against square of distance from center of rotation, r^2. Tll_{ex2} (0.05%) was analyzed by sedimentation

equilibrium on a Beckman model E analytical ultracentrifuge in aqueous solution (PBS) at 30,000 rpm (ω - 3.142 X 10^3 rad/sec) or in dissociating conditions (6 M guanidine hydrochloride) at 44,000 rpm (ω - 4.608 x 10^3 rad/sec). Data were obtained at 22°C (PBS) or 23°C (guanidine-hydrochloride). Assuming a partial specific volume of 0.725, the calculated molecular weight from the displayed slope and using the equation: MW - 4.606 RT x slope/[ω(1 - v^{bar} ω)] is 25,315 daltons in aqueous solution. Assuming a partial specific volume (denoted as v^{bar}) of 0.725 - 0.1 in 6 M guanidine hydrochloride (Richardson, N.E., et al., Biochem J. 135:87-92 (1973)), the calculated molecular weight is 24,736 daltons in dissociating conditions.

The expression of CD2 epitopes was investigated by immunoprecipitation analysis. The $T11_{ex2}$ molecule can be immunoprecipitated by both anti-$T11_1$ and a second monoclonal antibody to a different epitope termed anti-$T11_2$. However, $T11_{ex2}$ is not immunoprecipitated by the anti-CD2 antibody, anti-$T11_3$, which defines an activation specific epitope on CD2. Nevertheless, $T11_{ex2}$ was able to inhibit the binding of anti-$T11_3$-FITC to the $T11_3$ + Jurkat cell line at a concentration of 10 μm, implying its presence on $T11_{ex2}$ (data not shown). These results also suggest that the affinity of anti-$T11_3$ for its epitope is low.

Secondary structure predictions suggest the presence of both α-helical and β-sheet structure in the CD2 external domain (Clayton et al., Eur. J. Immunol. 17:1367-1370 (1987)). To more directly

predict secondary structural characteristics, the $T11_{ex2}$ molecule was evaluated by circular dichroism. Figure 6 shows the circular dichroism spectras of $T11_{ex2}$. Far ultraviolet circular dichroism spectra represent the average of 3-5 individual spectra with data taken at 0.5 nm wavelenght intervals in 10mM sodium phosphate, pH 7.2 A: spectrum at 25°C of untreated $T11_{ex2}$; B: spectrum of $T11_{ex2}$ reduced with 10 μM DTT and alkylated with 20μM iodacetamide; C: thermal denaturation of the sample in A; D: thermal denaturation of the sample in B.

As shown in Figure 6A, the far ultraviolet CD spectrum of $T11_{ex2}$ in 10 mM sodium phosphate shows a positive absorption maximum at about 200 nm ($\Delta\epsilon$ = 0.459), a negative minimum at 215 nm ($\Delta\epsilon$ = -1.94) and shoulder at 225 nm ($\Delta\epsilon$ = -1.0). When the $T11_{ex2}$ molecule is reduced by 50 mM DTT and subsequently alkylated with iodoacetamide, the CD spectrum is substantially altered, pointing to a role for disulfide bridges in stabilizing secondary and tertiary structure (Figure 6B). The fact that the spectrum of the non-reduced molecule reflects significant thermal denaturation at 80°C (Figure 6C) confirms that substantial secondary structure is present in soluble $T11_{ex2}$. As expected, the pattern after thermal denaturation is the same for the reduced as for the non-reduced molecule (compare Figures 6C and 6D).

In its overall pattern, the CD spectrum resembles that for Thy-1 (Campell et al., Nature 282:341-342 (1979)) which is a well-recognized

member of the immunoglobulin superfamily (Williams and Barclay, Annu. Rev. Immmunol. 6:381-405 (1988)) and is therefore predicted to consist entirely of β-sheet. However, the shoulder at 225 nm is absent from the Thy-1 profile. To obtain a more objective prediction of secondary structure from the CD spectrum, the digitalized absorption data (Table I) were deconvoluted according to the inverse matrix method of Compton and Johnson, Anal. Biochem. 155:155-167 (1986). The resulting predictions for proportions of secondary elements are: α-helix, 20%; anti-parallel β-sheet, 13%; parallel β-sheet, 9%, turn, 20%, other, 46%. Since none of the protein in the data sets used to determine the matrix values are homologous to CD2, the predicted fractions of secondary structure are only approximate. When the same CD data are deconvoluted by the method of Yang et al., Meth. Enzymol. 130:208-209 (1986a), 15% α and 40% β structures are predicted.

TABLE I

DIGITIZED PROTEIN CIRCULAR DICHROISM SPECTRUM FOR $T11_{ex2}$ FROM 184-260 nm at 2 nm INTERVALS

Wavelength	Δε	Wavelength	Δε
184	-1.49	224	-1.002
186	-1.50	226	-0.923
188	-1.345	228	-0.856
190	-1.176	230	-0.743
192	-1.026	232	-0.629
194	-0.284	234	-0.515
196	0.241	236	-0.426
198	0.411	238	-0.348
200	0.459	240	-0.282
202	0.360	242	-0.234
204	0.027	244	-0.195
206	-0.432	246	-0.151
208	-0.959	248	-0.112
210	-1.467	250	-0.089
212	-1.750	252	-0.077
214	-1.949	254	-0.062
216	-1.930	256	-0.044
218	-1.771	258	-0.018
220	-1.515	260	-0.018
222	-1.241		

Spectrum was taken in 10 mM sodium phosphate (pH 7.2) in a 1 mm cell at 22°C. Secondary structure predictions were calculated by taking dot products with inverse CD spectra for the five secondary

structure categories as described in Table 6 of Compton and Johnson, Appl. Biochem. 155:155-167 (1986). The calculated results are: α-helix, 20%; anti-parallel β-sheet, 13%; parallel β-sheet, 9%; turn, 20%; other, 46%. Units for $\Delta\epsilon$ are $M^{-1}cm^{-1}$.

To investigate the presence of protease-resistant domains in the $T11_{ex2}$ molecule, limited papain digestions were performed. Fifteen min. digestion at an enzyme:protein ratio of 1:250 at 37°C yields a major band at 15KD when analyzed by SDS-PAGE (data not shown). Longer digestion times cause partial disappearance of the 15KD band. Approximately 400 pmol of this 15KD material was blotted onto PVDF and sequenced, yielding 19 unambiguous residues corresponding to the CD2 amino-terminus. These results demonstrate that the carboxy region of $T11_{ex2}$ is much less resistant to papain digestion that its amino-terminal counterpart.

$T11_{ex2}$ inhibits sheep erythrocyte rosetting

The ability of $T11_{ex2}$ to interact with the CD2 ligand expressed on the surface of various cell types was investigated. The capacity of $T11_{ex2}$ to inhibit sheep erythrocyte rosetting with T lymphocytes was tested. Table II shows that rosetting is completely inhibited at concentrations of $T11_{ex2}$ greater than 5 μM; half-maximal inhibition occurs between 0.63 and 1.25 μM $T11_{ex2}$. Note that the anti-$T11_1$ (3T4-8B5) antibody abrogates rosetting at a concentration as low as 0.007 μM (Table II). This result suggests that any direct interaction between the soluble $T11_{ex2}$ molecule and the CD2 ligand is of relatively low affinity.

TABLE II

CONCENTRATION DEPENDENCE OF $T11_{ex2}$ INHIBITION OF SHEEP ERYTHROCYTE-HUMAN T CELL ROSETTES

Protein Added (μm)		Rosettes (% of Control)
None		100
$T11_{ex2}$	20	0
	10	0
	5	0
	2.5	4.8
	1.25	9.9
	0.63	122
	0.31	84
	0.16	116
	0.08	110
$T4_{ex1}$	20	66
	10	91
	5	96
	2.5	105
	1.25	90
	0.63	72
	0.31	110
	0.16	99
	0.08	116
Anti-CD2	0.7	0
	0.07	0
	0.006	0

For rosette formation, sheep erythrocytes were preincubated with soluble protein or antibody at the indicated concentrations for 1 h at 4°C followed by the addition of Jurkat cells, co-centrifugation and a further 1 h incubation at 4°C. Rosettes were counted in a hemacytometer. Control value was the fraction of rosette formation in the absence of added protein. Anti-CD2 was the anti-$T11_1$ antibody of 3T4-8B5.

$T11_{ex2}$ blocks the binding of anti-LFA-3 monoclonal antibody

To investigate whether $T11_{ex2}$ could interact with a previously defined ligand for CD2 on human cells (Selveraj et al., Nature 326:400-403 (1987), its ability to block the binding of monoclonal anti-LFA-3 antibody TS9/2 (Sanchez-Madrid et al., Proc. Natl. Acad. Sci. USA 79:7489-7493 (1982) to LFA-3 bearing cells was tested. Specifically, anti-LFA-3 reactivity was measured by FACS analysis on the human B lymphoblastoid line JY, which expresses high levels of LFA-3. As shown in Table III, preincubation of JY cells with soluble CD4 does not affect this staining, whereas preincubation with 10 μM $T11_{ex2}$ causes a substantial decrease in observed fluorescence, reducing linear immunofluoroescence from channel 120 to 30.6. This represents a reduction of 85% of specific anti-LFA-3 reactivity (calculated after subtraction of the background fluorescence of 13.2 linear units). Significant blocking is also seen at 1 μM but not at 0.1 μM. Addition of the control soluble CD4 $T4_{ex1}$ protein has no effect on anti-LFA-3 binding. Thus, by this measure, as well as by inhibition of sheep erythrocyte rosetting, the affinity of $T11_{ex2}$ for its ligand is apparently in the micromolar range.

TABLE III

CONCENTRATION DEPENDENCE OF $T11_{ex2}$ INHIBITION OF MONOCLONAL ANTI-LFA-3 ANTIBODY BINDING

Protein Added	Anti-LFA-3 Antibody Added	Mean Fluorescense Intensity
$T11_{ex2}$:		
300 μg/ml (10 μM)	+	30.6
30 μg/ml (1 μM)	+	66.1
3 μg/ml (0.1 μM)	+	109.1
0.3 μg/ml (0.01 μM)	+	119.8
$T4_{ex1}$:		
300 μg/ml	+	125.1
30 μg/ml	+	120.2
3 μg/ml	+	117.6
0.3 μg/ml	+	116.9
None	+	123.7
	-	13.2

Mean fluorescence intensities were obtained on a linear scale from 0-250.

$T11_{ex2}$ binds to LFA-3 on human β lymphoblastoid cells

Since $T11_{ex2}$ clearly inhibits sheep erythrocyte rosetting and blocks the binding of anti-LFA-3 antibody, we determined whether specific, saturable binding of $T11_{ex2}$ to human cells bearing LFA-3 could be detected. Two types of binding assays were employed. In the first, increasing amounts of unlabeled $T11_{ex2}$ was added to a mixture of the JY B lymphoblastoid cell line plus a constant amount of ^{125}I labeled $T11_{ex2}$ (ligand).

Figure 7 shows the competitive inhibition of radioiodinated $T11_{ex2}$ binding to JY cells. 5×10^6 cpm radiolabelled $T11_{ex2}$ (2.8×10^8 cpm/nmole) was added at 0.1 μM to 1.8×10^6 JY cells overlayed onto 0.2 ml of a 1.5:1 mixture of dibutyl phthalate: dioctyl phthalate:dioctyl phthalate in 0.5 ml plastic tubes. Increasing concentrations of unlabeled $T11_{ex2}$ (closed circles) or $T4_{ex1}$ as a control (open circles) were added in a final volume of 200 μl in RPMI 1640/10% FCS. After 1 h incubation at 4°C, tubes were spun, cell pellets severed and bound radioactivity was determined. To one tube anti-LFA-3 antibody TS2/9 was added at 50 μg/ml as a determination of non-specific binding. Specific cpm are the total cpm minus cpm bound in the presence of anti-LFA-3 (9070 cpm). Binding in the presence on control anti-HLA monoclonal antibody W6/32 is also shown (triangle).

As shown in Figure 7, binding of radiolabeled $T11_{ex2}$ is progessively inhibited by the addition of

increasing amounts of unlabeled molecules. Half-maximal inhibition occurs at about 0.5 μM $T11_{ex2}$. In contrast and as expected, addition of soluble CD4 $T4_{ex1}$ protein has no effect on ^{125}I-labeled $T11_{ex2}$ binding to JY.

As a second measure of binding, increasing amounts of labeled $T11_{ex2}$ were added to a constant number of JY cells. Figure 8 shows the saturation binding of $T11_{ex2}$ to JY cells and Scatchard analysis: Increasing concentrations of radiolabeled $T11_{ex2}$ (1.31×10^7 cpm/nmole) were added to 2.6×10^6 JY cells in the presence or absence of 50 μg/ml anti-LFA-3 antibody to determine nonspecific binding. Binding was carried out as above and the dissociation constant determined by Scatchard analysis after subtraction of nonspecific binding determined in the presence of anti-LFA-3. Top: total binding (open circles) and specific binding (closed circles). Botton: Scatchard analysis: correlation coefficient = 0.98; -1/slope - K_d = 0.4 μM.

As shown in Figure 8 (top), specific binding is saturable. These saturation binding data were transformed by Scatchard analysis (Figure 8, bottom). Specific $T11_{ex2}$ binding is saturated at about 300×10^3 molecules per cell. The Scarchard plot yields an estimated dissociation constant of 0.4 μM for the $T11_{ex2}$-LFA-3 interaction. Note that although a single incubation period (1 h at 4°C) of cells with ligand was used in these experiments, kinetic analysis demonstrates that maximal $T11_{ex2}$-LFA-3 binding occurs within 5 min.

Conclusion

We have shown that a soluble, monomeric extracellular segment CD2 molecule, termed $T11_{ex2}$, carries epitopes recognized by antibodies against the native surface CD2 structure on resting T lymphocytes and interacts specifically with the surface-bound CD2 ligand, LFA-3. The measured dissociation constant for this interaction is 0.4 μM, implying a low affinity relative to hormone receptor-ligand interactions (i.e., for IL-2 K_d = 10^{-11} M)(Smith, 1984) but equivalent to that of primary antibody responses (K_d = 10^{-5} to 10^{-6}) (Eisen and Siskind, 1964). The $T11_{ex2}$ molecule gives rise to a proteolytically-resistant 15KD amino-terminal papain fragment, suggesting that the amino-terminal ~100 amino acid residues comprise a stable, well-folded domain corresponding to a polypeptide encoded by the first extracellular exon. In contrast, despite disulfide linkages, the carboxy-terminal encoded extracellular domain is labile to papain.

Deposit

The virus, AcNP/$T11_{ex2}$ has been deposited at the American Type Culture Collection in Rockville, Maryland on January 19, 1989, and assigned the ATCC Accession Number _____.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiment of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

1. A soluble peptide having a lymphocyte function-associated antigen 3 (LFA-3) binding domain and antigenic epitopes recognized by antibodies raised against native CD2 on the surface of human T cells; the peptide being capable of binding LFA-3 and forming at least two intramolecular disulfide bonds.

2. A soluble peptide of Claim 1 wherein the antigenic epitopes are $T11_1$, $T11_2$ and $T11_3$.

3. A soluble peptide of Claim 2 wherein the peptide exists as a monomer in aqueous medium.

4. An isolated soluble peptide having an amino acid sequence encoded by the two extracellular segment exons of the gene encoding human CD2 glycoprotein, the peptide being capable of binding LFA-3, inhibiting CD2-mediated T cell activation and reacting with antibodies raised against native CD2 on the surface of human T cells.

5. A soluble peptide of Claim 4 wherein the peptide exists as a monomer in aqueous medium.

6. The soluble peptide of Claim 4 having the amino acid sequence:

Lys Glu Ile Thr Asn Ala Leu Glu Thr Trp
Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp
Ile Pro Ser Phe Gln Met Ser Asp Asp Ile
Asp Asp Ile Lys Trp Glu Lys Thr Ser Asp
Lys Lys Lys Ile Ala Gln Phe Arg Lys Glu
Lys Glu Thr Phe Lys Glu Lys Asp Thr Tyr
Lys Leu Phe Lys Asn Gly Thr Leu Lys Ile
Lys His Leu Lys Thr Asp Asp Gln Asp Ile
Tyr Lys Val Ser Ile Tyr Asp Thr Lys Gly
Lys Asn Val Leu Glu Lys Ile Phe Asp Leu
Lys Ile Gln Glu Arg Val Ser Lys Pro Lys
Ile Ser Trp Thr Cys Ile Asn Thr Thr Leu
Thr Cys Glu Val Met Asn Gly Thr Asp Pro
Glu Leu Asn Leu Tyr Gln Asp Gly Lys His
Leu Lys Leu Ser Gln Arg Val Ile Thr His
Lys Trp Thr Thr Ser Leu Ser Ala Lys Phe
Lys Cys Thr Ala Gly Asn Lys Val Ser Lys
Glu Ser Ser Val Glu Pro Val Ser Cys Pro
Glu Lys

and modifications of the peptide in which amino acid residues have been deleted, inserted or substituted without essentially detracting from the properties thereof.

7. The soluble peptide of Claim 6 comprising Tll_1, Tll_2 and Tll_3 epitopes localized on the peptide.

8. The soluble peptide of Claim 7 having the LFA-3 binding domain.

9. An isolated DNA sequence encoding a soluble peptide having a lymphocyte function-associated antigen 3 binding domain and antigenic epitopes recognized by antibodies raised against native CD2 on the surface of human T cells; the peptide being capable of binding LFA-3 and forming at least two intramolecular disulfide bonds.

10. An isolated DNA sequence encoding a soluble peptide having an amino acid sequence encoded by the two extracellular segment exons of the gene encoding human CD2 glycoprotein, the peptide being capable of binding LFA-3, inhibiting CD2-mediated T cell activation and reacting with antibodies raised against native CD2 on the surface of human T cells.

11. An isolated DNA sequence encoding the amino acid sequence of Claim 6 or substantial coding equivalents thereof.

12. An expression vector having an isolated DNA sequence as claimed in Claim 9.

13. An expression vector having the isolated DNA sequence as claimed in Claim 10.

14. An expression vector having an isolated DNA sequence encoding the amino acid sequence of Claim 5.

15. An expression vector of Claim 9 wherein the vector is a baculovirus transfer vector.

16. A cell transformed with the expression vector of Claim 12.

17. A cell transformed with the expression vector of Claim 13.

18. A cell transformed with the expression vector of Claim 14.

19. A plasmid having the ATCC Accession Number _____.

20. A method of inhibiting T cell activation comprising the step of administering to a patient a soluble peptide having a lymphocyte function-associated antigen 3 binding domain and antigenic epitopes recognized by antibodies raised against native CD2 on the surface of human T cells; the peptide being capable of binding LFA-3 and forming at least two intramolecular disulfide bonds.

21. A method of Claim 20 wherein the soluble peptide is administered intravenously.

22. A method of inhibiting T cell activation comprising the step of administering to a patient a soluble peptide having an amino acid sequence encoded by the two extracellular segment exons of the gene encoding human CD2 glycoprotein, the peptide being capable of binding LFA-3, inhibiting T cell activation and reacting with antibodies raised against the native CD2 protein on the surface of human T cells.

23. A method of Claim 22 wherein the soluble peptide comprise the amino acid sequence:

Lys Glu Ile Thr Asn Ala Leu Glu Thr Trp
Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp
Ile Pro Ser Phe Gln Met Ser Asp Asp Ile
Asp Asp Ile Lys Trp Glu Lys Thr Ser Asp
Lys Lys Lys Ile Ala Gln Phe Arg Lys Glu
Lys Glu Thr Phe Lys Glu Lys Asp Thr Tyr
Lys Leu Phe Lys Asn Gly Thr Leu Lys Ile
Lys His Leu Lys Thr Asp Asp Gln Asp Ile
Tyr Lys Val Ser Ile Tyr Asp Thr Lys Gly
Lys Asn Val Leu Glu Lys Ile Phe Asp Leu
Lys Ile Gln Glu Arg Val Ser Lys Pro Lys
Ile Ser Trp Thr Cys Ile Asn Thr Thr Leu
Thr Cys Glu Val Met Asn Gly Thr Asp Pro
Glu Leu Asn Leu Tyr Gln Asp Gly Lys His
Leu Lys Leu Ser Gln Arg Val Ile Thr His
Lys Trp Thr Thr Ser Leu Ser Ala Lys Phe
Lys Cys Thr Ala Gly Asn Lys Val Ser Lys
Glu Ser Ser Val Glu Pro Val Ser Cys Pro
Glu Lys

and modifications of the peptide in which amino acid residues have been deleted, inserted or substituted without essentially detracting from the properties thereof.

24. A method of Claim 23 wherein the soluble peptide is administered intravenously.

-24	Met	Ser	Phe	Pro	Cys	Lys	Phe	Val	Ala	Ser	Phe	Leu	Leu	Ile	Phe	L
- 9	Asn	Val	Ser	Ser	Lys	G										
										+1						
						ly	Ala	Val	Ser	Lys	Glu	Ile	Thr	Asn	Ala	
7	Leu	Glu	Thr	Trp	Gly	Ala	Leu	Gly	Gln	Asp	Ile	Asn	Leu	Asp	Ile	
22	Pro	Ser	Phe	Gln	Met	Ser	Asp	Asp	Ile	Asp	Asp	Ile	Lys	Trp	Glu	
37	Lys	Thr	Ser	Asp	Lys	Lys	Lys	Ile	Ala	Gln	Phe	Arg	Lys	Glu	Lys	ExI
52	Glu	Thr	Phe	Lys	Glu	Lys	Asp	Thr	Tyr	Lys	Leu	Phe	Lys	Asn	Gly	
67	Thr	Leu	Lys	Ile	Lys	His	Leu	Lys	Thr	Asp	Asp	Gln	Asp	Ile	Tyr	
82	Lys	Val	Ser	Ile	Tyr	Asp	Thr	Lys	Gly	Lys	Asn	Val	Leu	Glu	Lys	
97	Ile	Phe	Asp	Leu	Lys	Ile	Gln	G								
								lu	Arg	Val	Ser	Lys	Pro	Lys	Ile	
112	Ser	Trp	Thr	Cys	Ile	Asn	Thr	Thr	Leu	Thr	Cys	Glu	Val	Met	Asn	
127	Gly	Thr	Asp	Pro	Glu	Leu	Asn	Leu	Tyr	Gln	Asp	Gly	Lys	His	Leu	Ex2
142	Lys	Leu	Ser	Gln	Arg	Val	Ile	Thr	His	Lys	Trp	Thr	Thr	Ser	Leu	
157	Ser	Ala	Lys	Phe	Lys	Cys	Thr	Ala	Gly	Asn	Lys	Val	Ser	Lys	Glu	
172	Ser	Ser	Val	Glu	Pro	Val	Ser	Cys	Pro	G						
										lu	Lys	Gly	Leu	Asp	Ile	
187	Tyr	Leu	Ile	Ile	Gly	Ile	Cys	Gly	Gly	Gly	Ser	Leu	Leu	Met	Val	
202	Phe	Val	Ala	Leu	Leu	Val	Phe	Tyr	Ile	Thr	Lys	Arg	Lys	Lys	Gln	TM
217	Arg	Ser	Arg	Arg	Asn	A										
						sp	Glu	Glu	Leu	Glu	Thr	Arg	Ala	His	Arg	
232	Val	Ala	Thr	Glu	Glu	Arg	Gly	Arg	Lys	Pro	His	Gln	Ile	Pro	Ala	
247	Ser	Thr	Pro	Gln	Asn	Pro	Ala	Thr	Ser	Gln	His	Pro	Pro	Pro	Pro	
262	Pro	Gly	His	Arg	Ser	Gln	Ala	Pro	Ser	His	Arg	Pro	Pro	Pro	Pro	
277	Gly	His	Arg	Val	Gln	His	Gln	Pro	Gln	Lys	Arg	Pro	Pro	Ala	Pro	C
292	Ser	Gly	Thr	Gln	Val	His	Gln	Gln	Lys	Gly	Pro	Pro	Leu	Pro	Arg	
307	Pro	Arg	Val	Gln	Pro	Lys	Pro	Pro	His	Gly	Ala	Ala	Glu	Asn	Ser	
322	Leu	Ser	Pro	Ser	Ser	Asn										

FIG. 1

```
                                        38                  53
                                ATG AGC TTT CCA TGT AAA TTT GTA GCC AGC TTC
                                MET Ser Phe Pro Cys Lys Phe Val Ala Ser Phe    - 14

                  68                    83                  98
CTT CTG ATT TTC AAT GTT TCT TCC AAA GGT GCA GTC TCC AAA GAG ATT ACG AAT
Leu Leu Ile Phe Asn Val Ser Ser Lys Gly Ala Val Ser Lys Glu Ile Thr Asn    - 5

113                 128                 143                 158
GCC TTG GAA ACC TGG GGT GCC TTG GGT CAG GAC ATC AAC TTG GAC ATT CCT AGT
Ala Leu Glu Thr Trp Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp Ile Pro Ser   23

        173                 188                 203                 218
TTT CAA ATG AGT GAT GAT ATT GAC GAT ATA AAA TGG GAA AAA ACT TCA GAC AAG
Phe Gln MET Ser Asp Asp Ile Asp Asp Ile Lys Trp Glu Lys Thr Ser Asp Lys   41

                233                 248                 263
AAA AAG ATT GCA CAA TTC AGA AAA GAG AAA GAG ACT TTC AAG GAA AAA GAT ACA
Lys Lys Ile Ala Gln Phe Arg Lys Glu Lys Glu Thr Phe Lys Glu Lys Asp Thr   59

    278                 293                 308                 323
TAT AAG CTA TTT AAA AAT GGA ACT CTG AAA ATT AAG CAT CTG AAG ACC GAT GAT
Tyr Lys Leu Phe Lys Asn Gly Thr Leu Lys Ile Lys His Leu Lys Thr Asp Asp   77

            338                 353                 368
CAG GAT ATC TAT AAG GTA TCA ATA TAT GAT ACA AAA GGA AAA AAT GTG TTG GAA
Gln Asp Ile Tyr Lys Val Ser Ile Tyr Asp Thr Lys Gly Lys Asn Val Leu Glu   95

383                 398                 413                 428
AAA ATA TTT GAT TTG AAG ATT CAA GAG AGG GTC TCA AAA CCA AAG ATC TCC TGG
Lys Ile Phe Asp Leu Lys Ile Gln Glu Arg Val Ser Lys Pro Lys Ile Ser Trp  113

        443                 458                 473                 488
ACT TGT ATC AAC ACA ACC CTG ACC TGT GAG GTA ATG AAT GGA ACT GAC CCC GAA
Thr Cys Ile Asn Thr Thr Leu Thr Cys Glu Val MET Asn Gly Thr Asp Pro Glu  131

                503                 518                 533
TTA AAC CTG TAT CAA GAT GGG AAA CAT CTA AAA CTT TCT CAG AGG GTC ATC ACA
Leu Asn Leu Tyr Gln Asp Gly Lys His Leu Lys Leu Ser Gln Arg Val Ile Thr  149

    548                 563                 578                 593
CAC AAG TGG ACC ACC AGC CTG AGT GCA AAA TTC AAG TGC ACA GCA GGG AAC AAA
His Lys Trp Thr Thr Ser Leu Ser Ala Lys Phe Lys Cys Thr Ala Gly Asn Lys  167

            608                 623                 638
GTC AGC AAG GAA TCC AGT GTC GAG CCT GTC AGC TGT CCA GAG AAA GGT CTG GAC
Val Ser Lys Glu Ser Ser Val Glu Pro Val Ser Cys Pro Glu Lys Gly Leu Asp  185

653                 668                 683                 698
ATC TAT CTC ATC ATT GGC ATA TGT GGA GGA GGC AGC CTC TTG ATG GTC TTT GTG
Ile Tyr Leu Ile Ile Gly Ile Cys Gly Gly Gly Ser Leu Leu MET Val Phe Val  203

        713                 728                 743                 758
GCA CTG CTC GTT TTC TAT ATC ACC AAA AGG AAA AAA CAG AGG AGT CGG AGA AAT
Ala Leu Leu Val Phe Tyr Ile Thr Lys Arg Lys Lys Gln Arg Ser Arg Arg Asn  221

                773                 788                 803
GAT GAG GAG CTG GAG ACA AGA GCC CAC AGA GTA GCT ACT GAA GAA AGG GGC CGG
Asp Glu Glu Leu Glu Thr Arg Ala His Arg Val Ala Thr Glu Glu Arg Gly Arg  239
```

FIG. 1A

SUBSTITUTE SHEET

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      818                 833                 848                 863
AAG CCC CAC CAA ATT CCA GCT TCA ACC CCT CAG AAT CCA GCA ACT TCC CAA CAT
Lys Pro His Gln Ile Pro Ala Ser Thr Pro Gln Asn Pro Ala Thr Ser Gln His  257

            878                 893                 908
CCT CCT CCA CCA CCT GGT CAT CGT TCC CAG GCA CCT AGT CAT CGT CCC CCG CCT
Pro Pro Pro Pro Pro Gly His Arg Ser Gln Ala Pro Ser His Arg Pro Pro Pro  275

923                 938                 953                 968
CCT GGA CAC CGT GTT CAG CAC CAG CCT CAG AAG AGG CCT CCT GCT CCG TCG GGC
Pro Gly His Arg Val Gln His Gln Pro Gln Lys Arg Pro Pro Ala Pro Ser Gly  293

      983                 998                1013                1028
ACA CAA GTT CAC CAG CAG AAA GGC CCG CCC CTC CCC AGA CCT CGA GTT CAG CCA
Thr Gln Val His Gln Gln Lys Gly Pro Pro Leu Pro Arg Pro Arg Val Gln Pro  311

           1043                1058                1073
AAA CCT CCC CAT GGG GCA GCA GAA AAC TCA TTG TCC CCT TCC TCT AAT
Lys Pro Pro His Gly Ala Ala Glu Asn Ser Leu Ser Pro Ser Ser Asn          327
```

FIG. 1A (CONT)

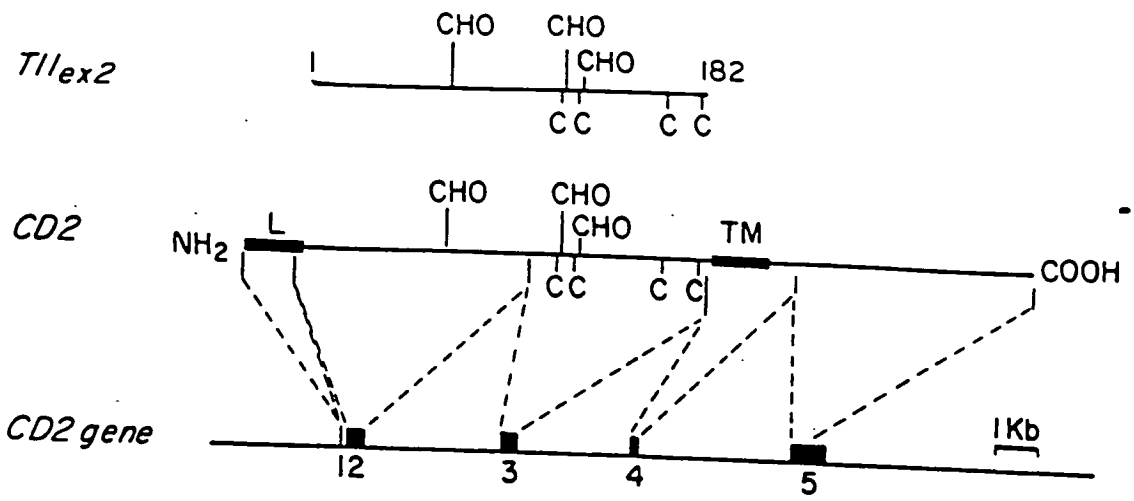

TIIex2
CHO
CHO
CHO
1
182
C C
C C
CD2
NH2
L
CHO
CHO
CHO
TM
COOH
C C
C C
CD2 gene
1Kb
1
2
3
4
5

FIG. 2

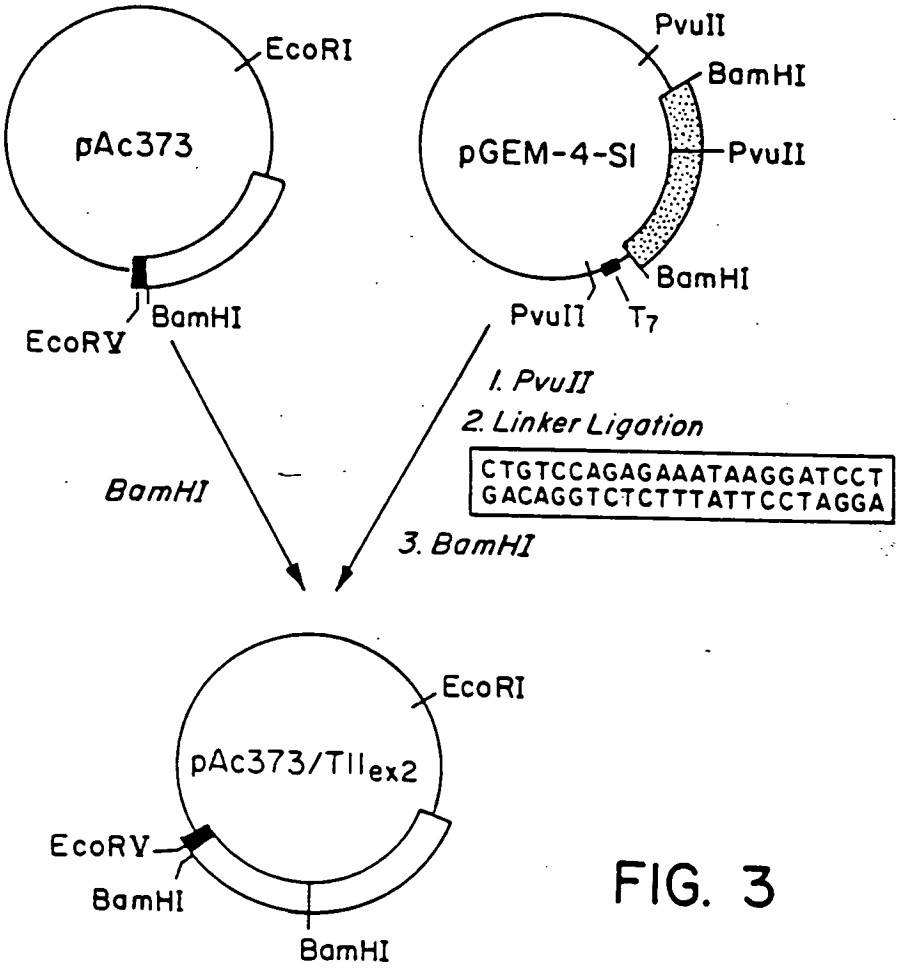

pAc373
EcoRI
EcoRV
BamHI
pGEM-4-SI
PvuII
BamHI
PvuII
BamHI
PvuII
T7
1. PvuII
2. Linker Ligation
CTGTCCAGAGAAATAAGGATCCT
GACAGGTCTCTTTATTCCTAGGA
BamHI
3. BamHI
pAc373/TIIex2
EcoRI
EcoRV
BamHI
BamHI

FIG. 3

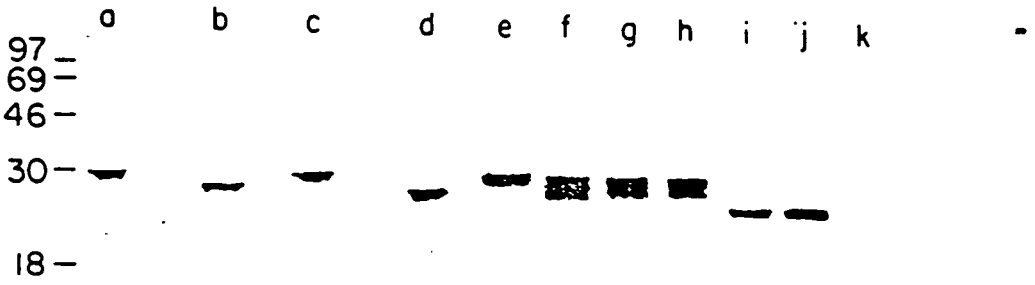

a
b
c
d
e
f
g
h
i
j
k
97
69
46
30
18

FIG. 4

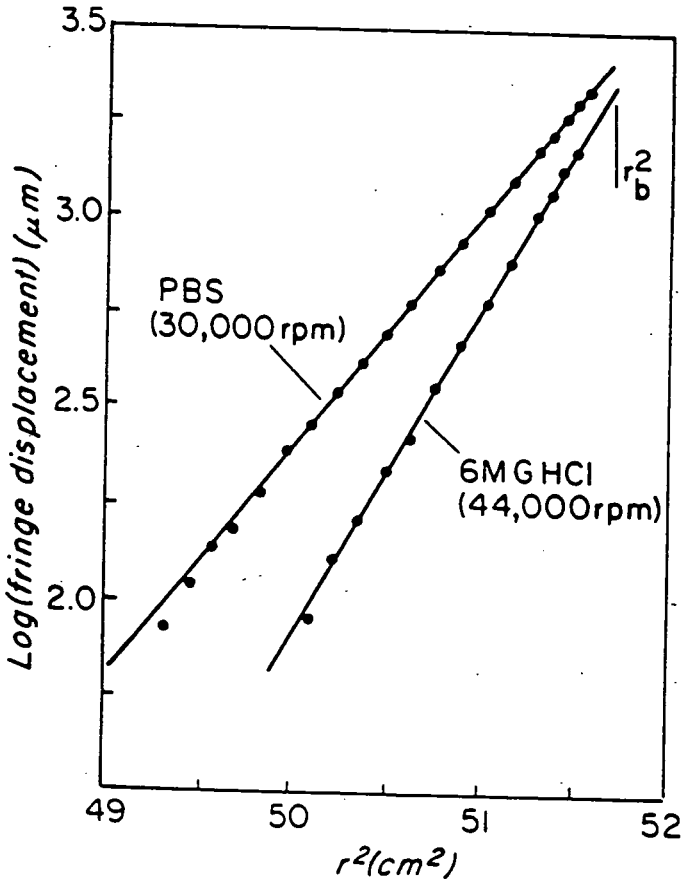

3.5
3.0
2.5
2.0
Log(fringe displacement) (μm)
PBS
(30,000 rpm)
6M G HCl
(44,000 rpm)
r_b^2
49
50
51
52
$r^2(cm^2)$

FIG. 5

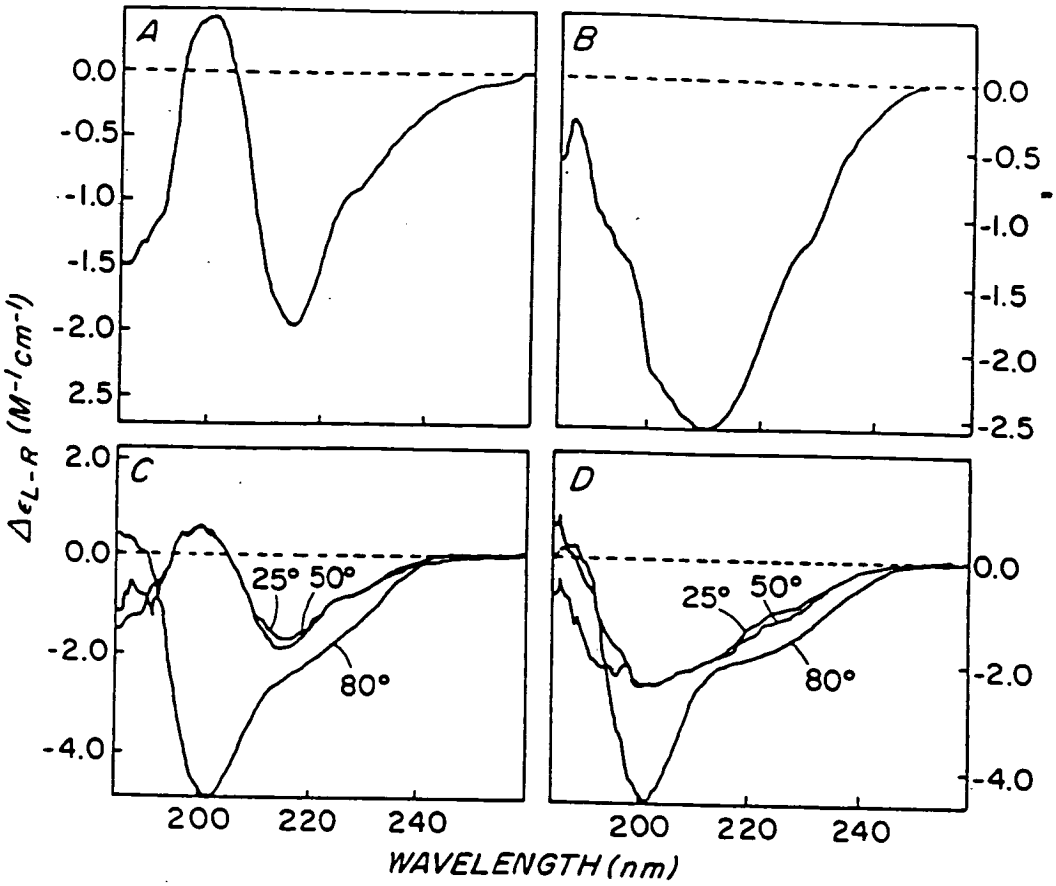

A
B
C
D
0.0
-0.5
-1.0
-1.5
-2.0
2.5
2.0
-4.0
25°
50°
80°
200
220
240
$\Delta\epsilon_{L-R}$ ($M^{-1}cm^{-1}$)
WAVELENGTH (nm)

FIG. 6

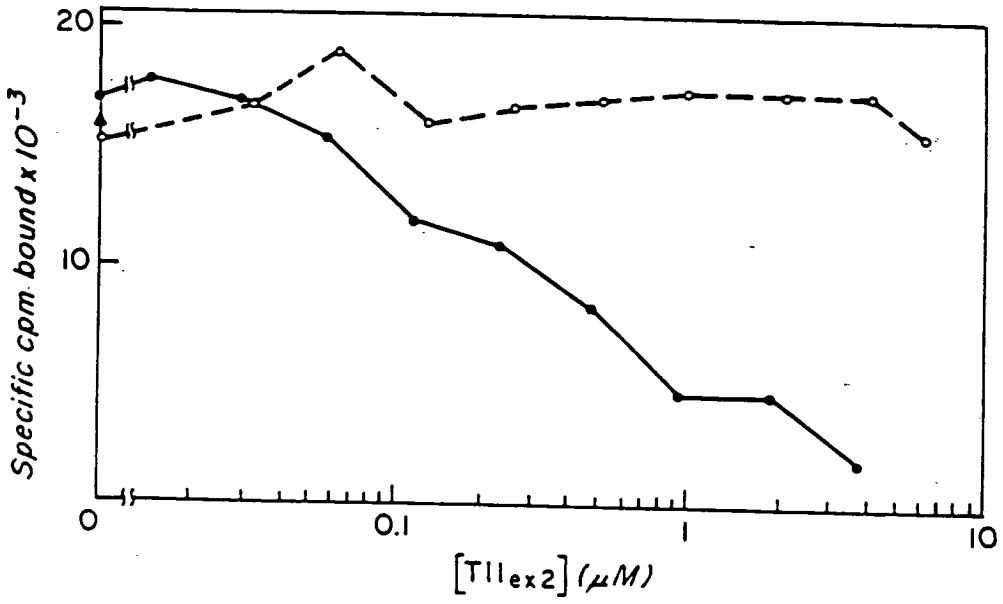
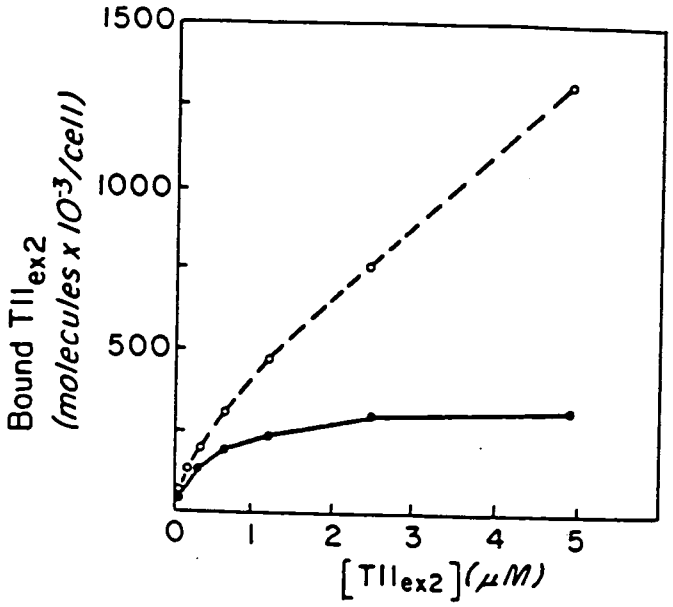

20
10
0
0.1
1
10
Specific cpm bound x 10^{-3}
[TIIex2] (μM)

FIG. 7


1500
1000
500
0
1
2
3
4
5
Bound TII$_{ex2}$ (molecules x 10^{-3}/cell)
[TII$_{ex2}$] (μM)

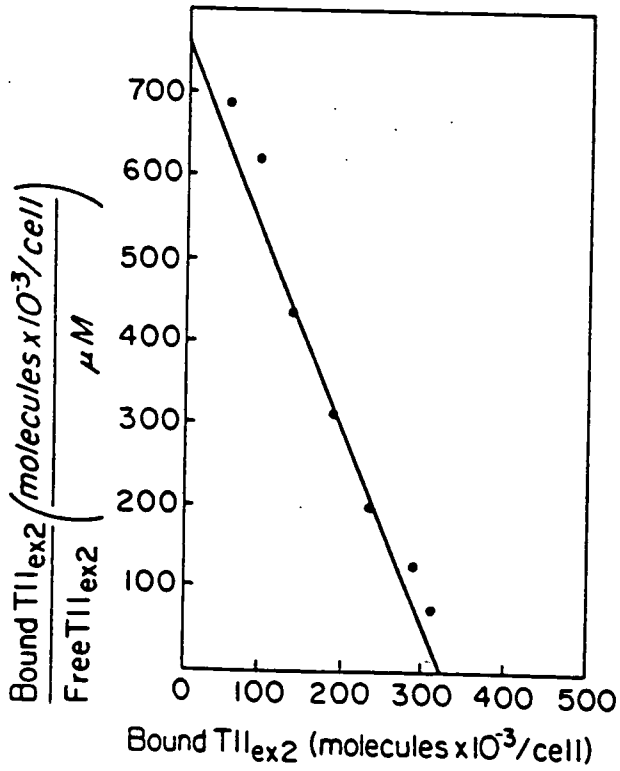

700
600
500
400
300
200
100
0
100
200
300
400
500
Bound TII$_{ex2}$ / Free TII$_{ex2}$ (molecules x 10^{-3}/cell / μM)
Bound TII$_{ex2}$ (molecules x 10^{-3}/cell)

FIG. 8

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 89/00218

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: C 12 N 15/00, C 12 P 21/04, C 07 K 13/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System [8]	Classification Symbols
IPC[4]	C 07 K, C 12 N, C 12 P

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [9]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	Chemical Abstracts, vol. 106, no. 21, 25 May 1987 (Columbus, Ohio, US) P. Selvaraj et al.: "The T lymphocyte glycoprotein CD2 binds the cell surface ligand LFA-3", see page 549, abstract no. 174287w & Nature (London) 1987, 326(6111) 400-3 --	1,4,9,10
A	Chemical Abstracts, vol. 107, no. 15, 12 October 1987 (Columbus, Ohio, US) P.H. Sayre et al.: "Molecular cloning and expression of T11 cDNAs reveal a receptor-like structure on human T lymphocytes", see page 177, abstract no. 128218x & Proc. Natl. Acad. Sci. U.S.A., 1987, 84(9) 2941-5 --	1-19

./.

* Special categories of cited documents: [14]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
10th August 1989	- 1 SEP 1989
International Searching Authority: EUROPEAN PATENT OFFICE	Signature of Authorized Officer: P.C.G. VAN DER PUTTEN

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Chemical Abstracts, vol. 108, no. 1, 4 January 1988 (Columbus, Ohio, US) A. Peterson et al.: "Monoclonal antibody and ligand binding sites of the T cell crythrocyte receptor (CD2)" see page 427, abstract no. 4386b & Nature (London) 1987, 329(6142), 842-6 --	1,4,7,8-10
T	Chemical Abstracts, vol. 110, no. 17, 24 April 1989 (Columbus, Ohio, US) P.H. Sayre et al.: "Structural and binding analysis of a two domain extracellular CD2 molecule" see page 549, abstract no. 152376e & Exp. Med. 1989, 169(3), 995-1009	1-19

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers XX, because they relate to subject matter not required to be searched by this Authority, namely:

XX Claims 20-22
See PCT Rule 39-1(iv):
Methods for treatement of the human or animal body, by means of surgery of therapy, as well as diagnostic methods.

2. [ ] Claim numbers ....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers ....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.